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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/500,118	09/14/2004	Henrik Semb	034005-008	7902

21839 7590 02/12/2007
BUCHANAN, INGERSOLL & ROONEY PC
POST OFFICE BOX 1404
ALEXANDRIA, VA 22313-1404

EXAMINER	
TON, THAIAN N	
ART UNIT	PAPER NUMBER
1632	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	02/12/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/500,118	SEMB ET AL.	
	Examiner	Art Unit	
	Thaian N. Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 January 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-61* is/are pending in the application.

4a) Of the above claim(s) *22-34 and 36-56* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-21,35 and 57-61* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *25 June 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7/13/06;1/27/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *Notice to Comply*.

DETAILED ACTION

Applicants' Remarks, filed 1/12/07, have been entered. Applicants' Preliminary Amendment, filed 6/25/04, has been entered. Claims 1-61 are pending; claims 22-34, 36-56 are withdrawn; claims 1-21, 35, 57-61 are under current examination.

Election/Restrictions

Applicant's election with traverse of Group I (claims 1-21, 35, 57-61) in the reply filed on 1/12/07 is acknowledged. The traversal is on the ground(s) that the inventions of Groups I-V are closely related and that a proper search of any of the claims should, by necessity, require a proper search of the others. Particularly, because all of the Groups, Applicants assert, are directed to the present stem cell line and differentiated cells from the same. Applicants argue that all of the claims can be search simultaneously, and that a duplicative search, with possibly inconsistent results, may occur if the restriction requirement is maintained. Furthermore, Applicants cite MNPEP §803, which is directed to the serious burden of search and examination. Applicants appear to argue that in the instant case there is no search burden. This is not found persuasive because of the following:.

The instant case is a 371, where search burden is not a requirement. See also, MPEP §801, which explicitly states that, "[A]pplications entering the National Stage under 35 U.S.C. 371 as a Designated or Elected Office in the U.S. Patent and Trademark Office is covered in Chapter 1800.". Wherein Chapter 1800, and specifically, 1893.03 (d), clearly states that, "Examiners are reminded that unity of invention (not restriction) practice is applicable in international applications (both Chapter I and II) and in national stage applications submitted under 35 U.S.C. 371." (emphasis added).

Thus, the Examiner has shown that Groups I-V designated in the Restriction requirement, mailed 12/13/06, do not relate to a single general

inventive concept under PCT Rule 13.1, and the Examiner has shown that the technical feature of Group I fails to be a special technical feature (see also, page 4of the Restriction requirement).

Thus, because it is determined that Groups I-V do not have unity of invention, the restriction requirement is deemed proper and is therefore made FINAL.

Claims 22-34, 36-56 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 1/12/07.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Information Disclosure Statement

Applicants' Information Disclosure Statements, filed 1/27/05 and 7/13/06 have been considered.

Sequence Compliance

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures. See page 9, lines 18-21.

Furthermore, Applicants must provide, as stated in the Notice to Comply, an substitute CRF, an substitute paper copy of the sequence listing and the appropriate statement accompanying this submission. Appropriate correction is required in order to constitute a proper response to this Office action. Applicant is requested to return a copy of the attached Notice to Comply with the response.

Priority

Receipt is acknowledged of papers submitted under 35 U.S.C. 119(a)-(d), which papers have been placed of record in the file. The Swedish Application, No. 0104471-8 (filed 12/28/01) has been received on 6/25/04.

Claim Objections

Claim 17 is objected to because of the following informalities: the claim does not have an appropriate article before the term "animal source". For example, "an animal source" would be grammatically appropriate. Claim 18 depends from claim 17. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-21, 35, 57-61 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for obtaining pluripotent human blastocyst-derived stem cell lines, wherein the inner cell mass cells are co-cultured on fibroblast feeder cells, and the blastocyst-derived stem cell line is propagated on fibroblast feeder cells does not

reasonably provide enablement for culturing inner cell mass cells or blastocyst-derived stem cell lines on any type of feeder cell layer. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the Invention. The claims are directed to methods of obtaining pluripotent human blastocyst-derived stem cell lines by using fertilized oocytes to obtain a blastocyst, co-culturing the blastocyst with feeder layers to establish one or more colonies of inner cell mass cells, isolating the inner cell mass cells by mechanical dissection, and co-culturing the inner cell mass cells with feeder cells to obtain a blastocyst-derived stem cell line. Specific embodiments are directed to further propagating the blastocyst-derived stem cell line.

Breadth of the claims. The breadth of the claims are directed to culturing inner cell mass cells (ICM) on any type of feeder cell to produce a blastocyst-derived stem cell line.

Guidance of the Specification/The Existence of Working Examples. The specification provides guidance with regard to the generating of human pluripotent blastocyst-derived stem cell lines. The specification contemplates utilizing feeder cells from any species, and particularly the feeder cells are embryonic feeder cells (see page 7, lines 4-8). The working examples in the specification are directed to culturing human blastocyst-derived stem cell lines on embryonic mouse fibroblasts (see Figures 3-5,, description on page

14, lines 26-28 and Example 4, for example). Accordingly, the specification provides guidance with regard to one type of feeder cell, fibroblast feeder cells.

State of the Art/Predictability of the Art. The state of the art of culturing of primate embryonic stem cells is such that culturing typically requires the presence of feeder cells. Thomson *et al.* discuss the difficulties in culturing pPS in feeder free conditions. Thomson *et al.* (**PNAS**, 92: 7844-7848, cited on Applicants' IDS, filed 1/27/05) teach the derivation of a cloned cell line from a rhesus monkey that remains undifferentiated when grown on mouse embryonic fibroblast feeder layers, but differentiate or die in the absence of the fibroblasts (see p. 7844, *Abstract*). Particularly, Thomson *et al.* state that in the absence of the feeder layers, soluble human leukemia inhibitory factor (LIF) fails to prevent the differentiation of the cells, and that the factors that fibroblasts produce to prevent the differentiation of the cells is yet unknown (see p. 7847, 1st column, 2nd paragraph). Thomson *et al.* further state that human inner cell mass-derived cells were cultured in the absence of feeder layers failed to survive beyond 2 passages (see p. 7848, 1st paragraph).

The requirement for human ES cells to be cultured on fibroblast feeder cells is additionally supported by Thomson *et al.* (**Tibtech**, 18:53-57 (2000), cited on Applicants' IDS, filed 1/27/05), who state that, "The critical factors produced by the fibroblast feeder layers that prevent differentiation of human ES cells and EG cells are unknown ... Human ES cells cultured in the presence of LIF and absence of fibroblasts uniformly differentiate or die within 1-2 weeks." See page 54, 2nd col., 2nd full ¶, emphasis added.

Further, the state of the art of culturing ES cells is unpredictable. Lim *et al.* [**Proteomics**, 2:1187-1203(2002)] teach the proteome analysis of conditioned medium from mouse embryonic fibroblast feeder layers to characterize the environment that supports the growth of undifferentiated

human ES cells, and to identify factors critical for their independent growth. See *Abstract.* Lim state that, "Despite many years of using mouse embryonic fibroblast cells as feeder support of human ES cells, it is still not clear what these cells for their clients. The interaction between these two cell types might take place *via* factors secreted into the medium or into extracellular matrix as well as through membrane-bound proteins." See p. 1188, 1st ¶. Lim teach that by utilizing proteomic analysis, unexpected results identify many known intracellular proteins, and that further analysis using serum-containing medium in the presence of ES cells, and using other cell types for feeder layers will be required. See p. 1203, 1st ¶, #4.

The Amount of Experimentation Necessary. The instant specification only provides guidance for culturing the human blastocyst-derived stem cells on fibroblast feeder layers. Furthermore, the state of the art teaches that it would not be predictable that any type feeder cell would be sufficient to support undifferentiated growth, as evidenced by Lim, who teach that the factors that maintain hES cells in an undifferentiated state, have yet to be identified. As specific factors produced by fibroblasts that support undifferentiated growth of hES cells have yet to be identified, it would not be predictable that any cell type, when used as claimed, would maintain the human blastocyst-derived stem cells in an undifferentiated state.

Accordingly, in view of the teachings of the state of the art with regard to the culturing of human embryonic stem cells, the lack of direction or guidance provided by the specification for culturing the undifferentiated human blastocyst-derived stem cells with any type of feeder cell, in order to maintain the hES cells in an undifferentiated state, it would have required undue experimentation for one of skill in the art to carry out the claimed methods.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-21, 35, 57-61 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-4 are confusing, because the method steps of the claims do not relate to the preamble, which refers to obtaining a human blastocyst derived stem cell line. The method steps do not relate to using human oocytes, or result in producing a human blastocyst stem cell line. It is suggested that the method steps of the claims reflect that the oocytes, blastocysts and resultant stem cell line reflect that they are human.

The term "less than about" in claims 9, 60 and 61 is a relative term which renders the claim indefinite. The term "less than about " is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The metes and bounds of this phrase are unclear, as to how much less, cells are required with regard to the limitation of the claim. Claim 10 depends from claim 9.

Claim 9 recites the limitation "the stem cells" in line 4. There is insufficient antecedent basis for this limitation in the claim. Line 8 is directed to propagating a blastocyst-derived stem cell line, thus, the term "the stem cells" lacks antecedent basis. Claim 10 similarly does not have antecedent basis for the term "the stem cells" in line 3 of the claim.

Claim 11 is confusing, because the metes and bounds of the term "feeder cells" cannot be determined. The claim refers to claim 7, which in turn, refers to claim 1. Claim 1 recites "feeder cells" in both step (ii) and step (iv). It is unclear which feeder cells the feeder cells of claim 11 refer to.

Claim 16 are similarly unclear because they refers to "the feeder cells" in line 2 of the claim.

Claim 14 is unclear because it recites the term "and/or". It is unclear if "and/or" is meant to further limit the claim. Claim 15 depends from claim 14.

Claim 18 is unclear because it recites that the feeder cells are of mouse or human origin. It is unclear which feeder cells recited in claim 17 – the feeder cells of step ii) or step iv). Because the two steps can have different feeder cells, or the same feeder cells, it is unclear which feeder cells from which step, claim 18 is intending to limit.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 21 is rejected under 35 U.S.C. 102(b) as being anticipated by Thomson *et al.* (Science, 282: 1145-1147, cited on Applicants' IDS, filed 7/13/05).

The claim is directed to a blastocyst-derived stem cells obtained by the method of claim 1.

Claim Interpretation. Claim 21 is product by process claims. Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. See In re Ludtke, supra. Whether the rejection is based on "inherency" under

35 USC 102, on "prima facie obviousness" under 35 USC 103, jointly or alternatively, the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products. In re Best, Bolton, and Shaw, 195 USPQ 430, 433 (CCPA 1977) citing In re Brown, 59 CCPA 1036, 459 F.2d 531, 173 USPQ 685 (1972). Further, see MPEP §2113, "Even though product-by process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process."

In the instant case, the claim is directed to a blastocyst-derived stem cell line. Thomson teach the derivation and culture of human ES cells, which are produced from human blastocysts. See Abstract.

Accordingly, Thomson teach the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 5-7, 12, 13, 16-20, 21, 57-59 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (U.S. Pat. No., 6,200,806 B1, issued March 13, 2001, hereafter referred to as 'Thomson (2001)') when taken with Thomson *et al.* (Science, 282: 1145-1147, 1998; cited on Applicants' IDS, filed 7/13/05, hereafter referred to as 'Thomson (1998)'), as evidenced by Stem Information (National Institutes of Health, Appendix C, pages 1-4, accessed online: http://stemcells.nih.gov/info/scireport/appendixC.asp January 31, 2007), when taken with Rijnders *et al.* (Hum. Reprod., 13(10): 2869-2873, 1998) and in further view of Lanzendorf *et al.*. (Fertility and Sterility, 76 (1): 132-137, July 2001).

The claims are directed to a method for obtaining a pluripotent human blastocyst-derived stem cell line, comprising i) using a fertilized oocyte, of grade 1 or 2 to obtain a blastocyst of grade A or B; ii) co-culturing the blastocyst with feeder cells to establish more colonies of inner cell mass cells; iii) isolating the inner cell mass cells by mechanical dissection and iv) co-culturing the inner cell mass cells with feeder cells to obtain a blastocyst derived stem cell line.

Thomson (2001) teach methods for the production of primate embryonic stem cells, including human ES cells. In particular, they teach the isolation of a blastocyst, isolating cells from the inner cell mass (ICM) of the blastocyst, and plating the ICM cells on embryonic feeder layers (see col. 4, lines 38-49). They teach that these methods can be used in deriving human ES cell lines (col. 7, lines 4-10). In particular, they teach isolating blastocysts from a primate, removing the zona pellucida using pronase, and then removal of the intact inner cell mass cells by gentle pipetting and plated on inactivated, irradiated embryonic fibroblasts (col. 8, lines 32-40). They teach that the dissociated cells are then replated on embryonic feeder layers, and cells demonstrating ES-like morphology are then individually selected and propagated. See col. 8, lines 50-59. They teach the co-culture of human blastocysts with human oviductal cells to produce expanded human blastocysts (see col. 9, lines 23-32). Thomson teach that their human ES cell line is stable (see issued claim 1). They further teach using mouse feeder cells, and particular, mouse STO cells (ATCC 56-X), which are irradiated mouse fibroblast feeder cells.

Thomson (1998) provide specific guidance to show that the production of human ES cells requires human embryos, which are produced by IVF (see p. 1147, col. 2, #6). With regard to claim 20, although Thomson (1998) teach that their cells have been passaged for more than 8 months, they do not specifically teach that the cells have a proliferation capacity, in an undifferentiated state, for more than 21 months, as required by part i) of claim 20. However, The NIH stem cell information provides evidence that the exact cell line described in the Thomson (1998) paper, the H9 cell line, has the proliferation capacity for more than 21 months, stating that, "[T]he H9 cell line has divided for nearly two years *in vitro*." See page 2, first sentence. Thus, this citation describes an inherent property of the cells, and shows that they have a proliferation capacity of over 21 months. Furthermore, Thomson

(1998) provide the various characteristics required by the claim 20, namely that the ES cell lines exhibited a normal karyotype (see page 1145, 2nd col.) (see part (ii) of the claim 20); had the developmental potential to form the derivatives of all three germ layers, both *in vitro* and *in vivo* (see Abstract, and page 1146, 1st col., 2nd full ¶) (part iii of the claim); exhibited appropriate markers, including expression of SSEA-3, SSEA-5, TRA-1-60, TRA-1-81 (p. 1145, 3rd col., last ¶), as required by part (iv) of the claim; did not express SSEA-1 (p. 1146, 1st col., 1st ¶); formed teratomas when injected into immunocompromised mice and is capable of differentiation (p. 1146, 2nd full ¶), as required by steps vi-vii of the claim.

Neither Thomson (2001), nor Thomson (1998) teach using a fertilized oocyte having a grade 1 or 2 to obtain a blastocyst of grade A or B, as recited in step (i) of claims 1-3. The instant specification defines Grade 1 fertilized oocytes as those which have even blastomers, with no fragments, and Grade 2 fertilized oocytes as those with <20% fragments (see page 4, lines 1-52); Grade A blastocysts are those with expanded distinct inner cell mass cells 5-7 days after fertilization, and Grade B blastocysts are not expanded but, otherwise like Grade A (p. 4, lines 10-11). Rijnders *et al.* provide specific guidance with regard to the identification of class I and class II embryos, which are defined similarly as the instant specification's definition (see p. 2870, 1st col., Embryo Pre-selection and Selection) and teach that class I and II embryos produced a higher percentage of blastocysts, and had less embryos that arrested in development of degenerated (see Abstract). Lazendorf *et al.* teach methods of identification of Grade 1 and 2 (analogous to the specification's definition of Grade A and B) blastocysts and they teach the hatching of the blastocysts (see page 134, 1st col.). They teach that some of the embryos, which were grade 1, hatched spontaneously, and that one blastocyst was grade 2, and required mechanical removal of the zona

pellucida (see p. 135, 1st col, Results, 1st ¶). They teach isolating inner cell masses from the blastocysts and producing embryonic stem cell lines.

Accordingly, it would have been obvious for one of ordinary skill in the art, to combine the teachings of Thomson (2001), Thomson (1998), who teach the production of human ES cell lines from human blastocysts, with the teachings of Rijnders and Lazendorf, with a reasonable expectation of success. One of ordinary skill would have been motivated to use the method of identifying fertilized oocytes/embryos of Grade 1 or 2, as taught by Rinjnders to increase the number of embryos that are capable of producing blastocysts. One of skill ordinary skill in the art would have been also motivated to use the methods, taught by Lazendorf *et al.* to identify blastocysts of Grade A or B, in order identify cells which have clearly defined inner cell masses, which would increase the probability of producing a successful ES cell line.

The combined teachings of Thomson (2001), Thomson (1998), Rinjnders and Lazendorf teach the claimed invention because the teach the general methods of selecting particular human fertilized oocytes or blastocysts for the production of human ES cell lines (claims 1-3), they teach that the blastocyst can be spontaneously hatched (claim 5); they teach that the stem cell line is stable (claim 6); wherein the cell line is propagated (claim 7, 57-59); wherein the zona pellucida of the blastocyst has been partially digested (claim 12); with a digestive agent (claim 13); wherein the feeder cells are embryonic feeder cells (claim 16); wherein the feeder cells in steps ii) and iv) are the same or different, and originate from an animal source (claim 17), particularly mouse (claim 18); wherein the feeder cells are mitotically inactivated (claim 19); the characteristics required by the stem cell line (claim 20); a blastocyst-derived stem cell line obtained by the method of claim 1 (claim 21).

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 4, 8-10, 60 and 61 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (2001) when taken with 'Thomson (1998) when taken with Rijnders *et al.* and in further view of Lanzendorf *et al.* as applied to claims 1-3, 5-7, 12, 13, 16-19, 21, 57-59 above, and further in view of Marshall *et al.* (**Methods in Molecular Biology**: *Isolation and Maintenance of Primate Embryonic Stem Cells* 158: 11-18, January 2001).

The claims further limit the above-described claims by reciting that the propagation of the blastocyst derived stem cell line is cultured with feeder cells of a density of less than about 60,000 cells/cm^2 (claim 4, 9); passaging the stem cell line every 4-5 days (claim 8); wherein the blastocyst stem cell line is cultured with feeder cells of a density of about 45,000 cells/cm^2 (claim 10); wherein the culturing uses feeders at a density of less than about 55,000 cells/cm^2 (claim 60), or less than about 50,000 cells/cm^2 (claim 61).

Thomson (2001), Thomson (1998); Rijnders and Lazendorf are described above. They do not teach the specific densities of the feeder cells required by the claims. However, Marshall discuss the isolation and maintenance of primate embryonic stem cells. They specific teach that the mouse embryonic fibroblasts, which are used as feeder cells, should be plated at 50,000 cells/cm^2 (see page 13, Section 3.1, #3). Thus, Marshall teach cell densities that are less than 60,000 (claims 4 and 9); "about" 45,000 cells/cm^2 (claim 10); less than 55,000 cells/cm^2 (claim 60) and "less than about 50,000 cells/cm^2 (claim 61). Furthermore, Marshall teach that the cells should be passaged four to six days after immunosurgery (see p. 13, 3.2, #9), and thus, fulfill the limitation of claim 8.

Claim Interpretation. The terms "less than about" and "about" are considered relative terms (see above, under 112, 2nd ¶), that are indefinite,

because the specification provides no specific guidance as to the range of "about", the art of Marshall, fulfills the limitations of these claims.

Accordingly, given the combined teachings of Thomson (2001), Thomson (1998); Rijnders, Lazendorf and Marshall, it would have been obvious for one of skill in the art to utilize the methods to produce blastocyst-derived stem cell lines, and passage the cells every 4-5 days, at a density of 50,000 cells/cm^2, as taught by Marshall, with a reasonable expectation of success. One of skill in the art would have been sufficiently motivated to utilize this amount of cells, as Marshall provide a specific protocol to maintain primate ES cells, and they state that because primate ES cells require "regular and meticulous attention to detail in all aspects of the culture process", one of skill in the art would turn to their protocol for direction and specific guidance with regard to the culture of primate ES cells. See page 12, 1st ¶.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijnders *et al.* and in further view of Lanzendorf *et al.* as applied to claims 1-3, 5-7, 12, 13, 16-19, 21, 57-59 above, and further in view of Conner (**Current Protocols in Molecular Biology**, 23.2.1-23.2.7, 2000).

The claim is directed to the method of claim 7, wherein the propagation of the blastocyst-derived stem cell line comprises passage of the feeder cells at the most 3 times.

Thomson (2001), Thomson (1998), Rijnders and Lanzendorf are detailed above. They do not specifically teach that the feeder cells are passaged only 3 times, at most. However, Conner provide guidance to the preparation of mouse embryo fibroblast feeder cells, which are used to

maintain human stem cells. See Thomson (2001), col. 8, lines 32-40; Thomson (1998), p. 1147, col. 2, #6. In particular, they suggest freezing the MEFs until use (p. 23.2.4, Freezing and Thawing MEFs). In particular, they teach the following:

> "The advantage of using MEFs is that they provide a more consistent source of feeder cells. Early passage cells with reproducible characteristics must be used because they rapidly lose their ability to divide. Long-term propagation of STO cells can lead to changes that result in characteristics that are less favorable for ES cell growth."
>
> See p. 23.2.6, Commentary, 2nd ¶, emphasis added.

Furthermore, Conner teach that MEFs are primary cells with limited mitotic potential, and that expanding the cells more may work, but the growth rate will decrease (see p. 23.2.7, 1st col., ¶2).

Accordingly, given the combined teachings, it would have been obvious to one of skill in the art, preparing human ES cells, and using embryonic fibroblasts as feeder cells, to use a early passage cell, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to use early passage embryonic fibroblasts, because Conner clearly teach that later passage cells lose the ability to divide, and can have changes in characteristics that are not conducive to ES cell growth. Although Conner do not specifically teach that the cells should be passaged less than 3 times, this would be well-within the knowledge of the skilled artisan that the less passages the embryonic fibroblasts are subjected to, the more conducive the feeder cells would be to maintain ES cells. Thus, using feeder cells that have been passaged less than 3 times would be well-within the skills of the ordinary artisan. Additionally, because Conner suggest that the MEFs should be frozen prior to use, this provides additional motivation and suggestion that the embryonic fibroblasts should not be subjected to many passages.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijnders *et al.* and in further view of Lanzendorf *et al.* as applied to claims 1-3, 5-7, 12, 13, 16-19, 21, 57-59 above, and in further view of Gardner *et al.* (Hum Reprod. 1998,_Jun;13 Suppl 3:148-60; . **Culture of viable human blastocysts in defined sequential serum-free media**), when taken with Gardner (**Hum. Reprod.**, 14(10):2575-2580, 1999).

The claims further limit claim 1, wherein steps ii or step iv) are performed in an agent that approves the attachments of the blastocysts and/or the inner cell mass to feeder cells, and particularly, where the agent is hyaluronic acid.

Thomson (2001), Thomson (1998), Rijnders and Lanzendorf are detailed above. They do not specifically teach culturing the blastocysts of step ii) or the inner cell mass of step iv) of claim in the presence of an agent, such as hyaluronic acid, to improve attachment of blastocysts. However, prior to the time of the claimed ivnetnion, Gardner teach that culturing of blastocysts in hyaluronic acid (hyaluronate) supports an increase in the implantation of blastocysts in IVF. See Abstract. Gardner teach that culturing human embryos in hyaluronate supports a significantly higher implantation rate (see page 155, 1st ¶); and that hyaluronate appears to be involved in the attachment of the blastocyst (see p. 155, 2nd ¶). Although Gardner's techniques are used in producing blastocysts that would be used in IVF, the fact that they show an improvement in attachment of the blastocyst is significant. They teach that studies in mice and cattle show that there is a relationship between the rate and normality of nutrient utilization and developmental potential, and that conventional embryo culture causes

significant trauma in the developing embryo. Gardner (1999) provides teachings to show that, in mice, blastocyst cell numbers and overall development increased when embryos were cultured in hyaluronan. See Abstract.

Accordingly, in view of the combined teachings, it would have been obvious for one of skill in the art to modify the methods of Thomson (2001), Thomson (1998), Rijnders and Lanzendorf, by culturing either the blastocyst of step ii) or the ICM cells of step iv) in a culture medium that contained hyaluronic acid, with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to make this modification, as Gardner and Gardner (1999) provide guidance to show that culture medium that contains hyaluronic acid increases attachment of the blastocyst.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (U.S. Pat. No., 6,200,806 B1, issued March 13, 2001, cited above) when taken with Stratagene Catalog, 1988, p. 39.

The claim is directed to a kit for performing the method of claim 1, comprising human blastocysts with an intact zona pellucida or spontaneously hatched blastocysts, and at least two of the following components in separate compartments: hyaluronic acid, pronase, BS-cell medium, and human or mouse embryonic feeder.

Thomson teach methods for the production of primate embryonic stem cells, including human ES cells. In particular, they teach the isolation of a blastocyst, isolating cells from the inner cell mass (ICM) of the blastocyst, and plating the ICM cells on embryonic feeder layers (see col. 4, lines 38-49). They teach that these methods can be used in deriving human ES cell lines (col. 7, lines 4-10). In particular, they teach isolating blastocysts from a

primate, removing the zona pellucida using pronase, and then removal of the intact inner cell mass cells by gentle pipetting and plated on inactivated, irradiated embryonic fibroblasts (col. 8, lines 32-40). They further teach using mouse feeder cells, and particular, mouse STO cells (ATCC 56-X), which are irradiated mouse fibroblast feeder cells. Thus, Thomson teach the components that are necessary in order to produce human ES cells, including the claimed embodiments of human blastocysts, pronase, and mouse embryonic feeders.

Although Thomson teaches the various components required by the claims, and that one of skill in the art would know that each of the components would be in separate compartments prior to the culturing of the ES cells, the Strategene catalog provides guidance to production of a kit. Although the Strategene catalog provides production to a gene characterization kit, one of skill in the art would recognize that putting components to produce human ES cells in a kit would have been obvious and with a reasonable expectation of success.

Accordingly, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine the method of Thomson into a kit format as discussed by Stratagene catalog since the Stratagene catalog teaches a motivation for combining reagents of use in an assay into a kit, "Each kit provides two services: 1) a variety of different reagents have been assembled and pre-mixed specifically for a defined set of experiments. Thus one need not purchase gram quantitites of 10 different reagents, each of which is needed in only microgram amounts, when beginning a series of experiments. When one considers all of the unused chemicals that typically accumulate in weighing rooms, desiccators, and freezers, one quickly realizes that it is actually far more expensive for a small number of users to prepare most buffer solutions from the basic reagents. Stratagene provides only the quantities you will actually need, premixed and

tested. In actuality, the kit format saves money and resources for everyone by dramatically reducing waste. 2) The other service provided in a kit is quality control" (page 39, column 1).

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Thaian N. Ton whose telephone number is (571) 272-0736. The Examiner can normally be reached on Monday through Thursday from 7:00 to 5:00 (Eastern Standard Time). Should the Examiner be unavailable, inquiries should be directed to Peter Paras, SPE of Art Unit 1632, at (571) 272-4517. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the Official Fax at (571) 273-8300. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

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thaian ton

THAIAN N. TON
PATENT EXAMINER

Application No.: 10/500,118

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

[X] 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.

[ ] 2. This application does not contain, as a separate part of the disclosure on paper copy, a Sequence Listing as required by 37 C.F.R. 1.821(c).

[ ] 3. A copy of the Sequence Listing in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).

[ ] 4. A copy of the Sequence Listing in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up Raw Sequence Listing.

[ ] 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).

[ ] 6. The paper copy of the Sequence Listing is not the same as the computer readable from of the Sequence Listing as required by 37 C.F.R. 1.821(e).

[ ] 7. Other:

Applicant Must Provide:

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[X] An initial or substitute paper copy of the Sequence Listing, as well as an amendment directing its entry into the specification.

[X] A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

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